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US EPAs R & D Activities Relevant to the ICCVAM Five Year Plan

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Office of Research and Development
National Center for Computational Toxicology

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Key Points

- ORD's mission is to lead the translation of scientific advances to address problems of national and international importance relative to protecting human health and the environment
- Multiple program offices within EPA recognize that the current methods for assessing chemical hazard and risk are insufficient for their tasks
  - *Legislation such as the new "Kid's Safe Chemicals Act" and FQPA in the U.S. and REACH in the EU highlight the problem*
- Recent advances in biology and computer sciences are enabling research that could not have been anticipated even 10 years ago.
- The transformation in toxicology necessitates an active research program within ORD and strategic staffing in the Program Offices
- ORD foresaw the emergence of computational toxicology, and its investment is now recognized internationally as the leading edge of change
  - *Requires integrated, multidisciplinary effort over prolonged period*

Administrator Jackson's Priorities

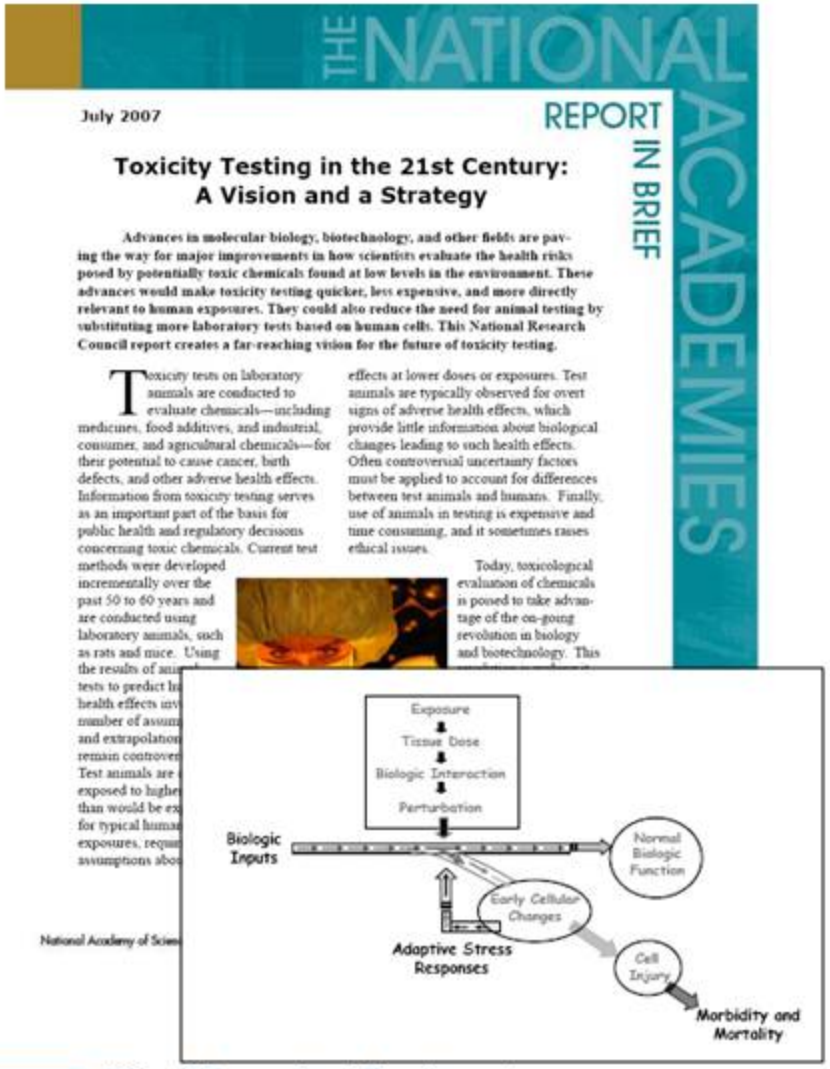
- Reducing Greenhouse Gas Emissions
- Improving Air Quality
- Managing Chemical Risks
  - "More that 30 years after Congress enacted the Toxic Substances Control Act, it is clear that we are not doing an adequate job of assessing and managing risks of chemicals in consumer products, the workplace and the environment. It is now time to revise and strengthen EPAs chemicals management and risk assessment programs"
  - "…we must be sensitive to the burdens pollution has placed on vulnerable subpopulations, including children, the elderly, the poor and all others who are at particular risk to threats to health and the environment. We must seek their full partnership in the greater aim of identifying and eliminating the sources of pollution in their neighborhoods, schools and homes."
- Cleaning up Hazardous Waster Sites
- Protecting America's Water

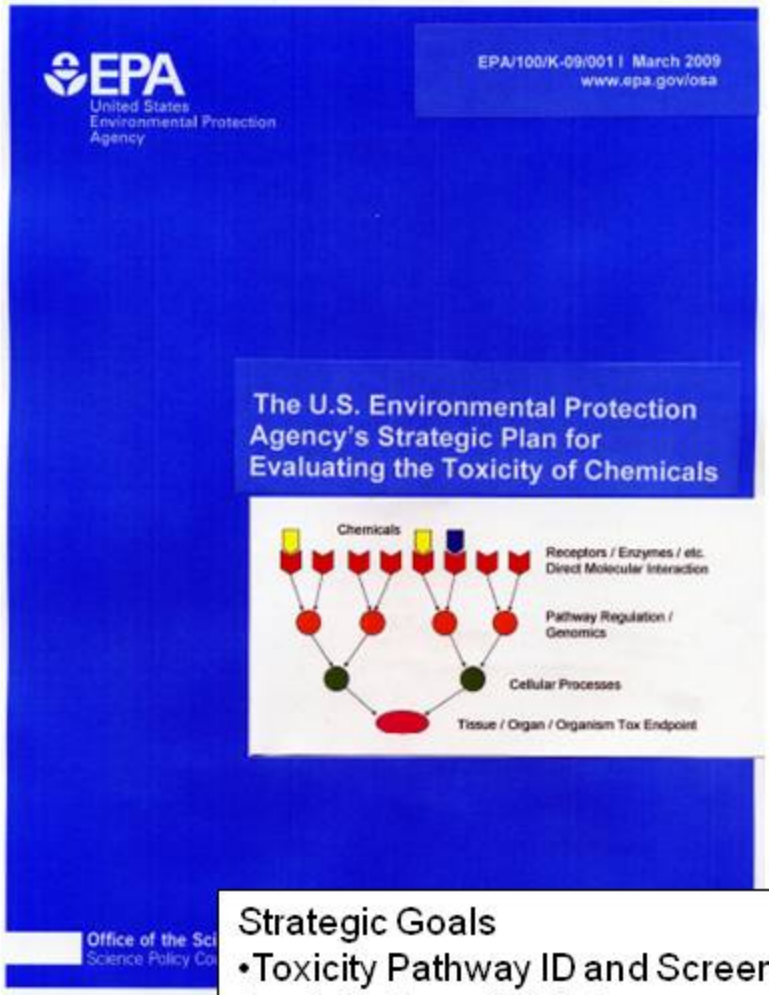
Source: Memo to EPA Employees, January 23, 2009
http://www.epa.gov/administrator/memotoemployees.html

NAS/NRC Consultations


2007	Toxicity Testing in the 21st Century: A Vision and a Strategy
2007	Applications of Toxicogenomic Technologies to Predictive Toxicology and Risk Assessment
2008	Phthalates and Cumulative Risk Assessment
2008	Science and Decisions-Advancing Risk Assessment
2009	Toxicity Pathway-Based Risk Assessment: Preparing for Paradigm Change, May 11-13, 2009

Transforming Toxicology





Strategic Goals
- Toxicity Pathway ID and Screening
- Toxicity Based Risk Assessment
- Institutional Transition

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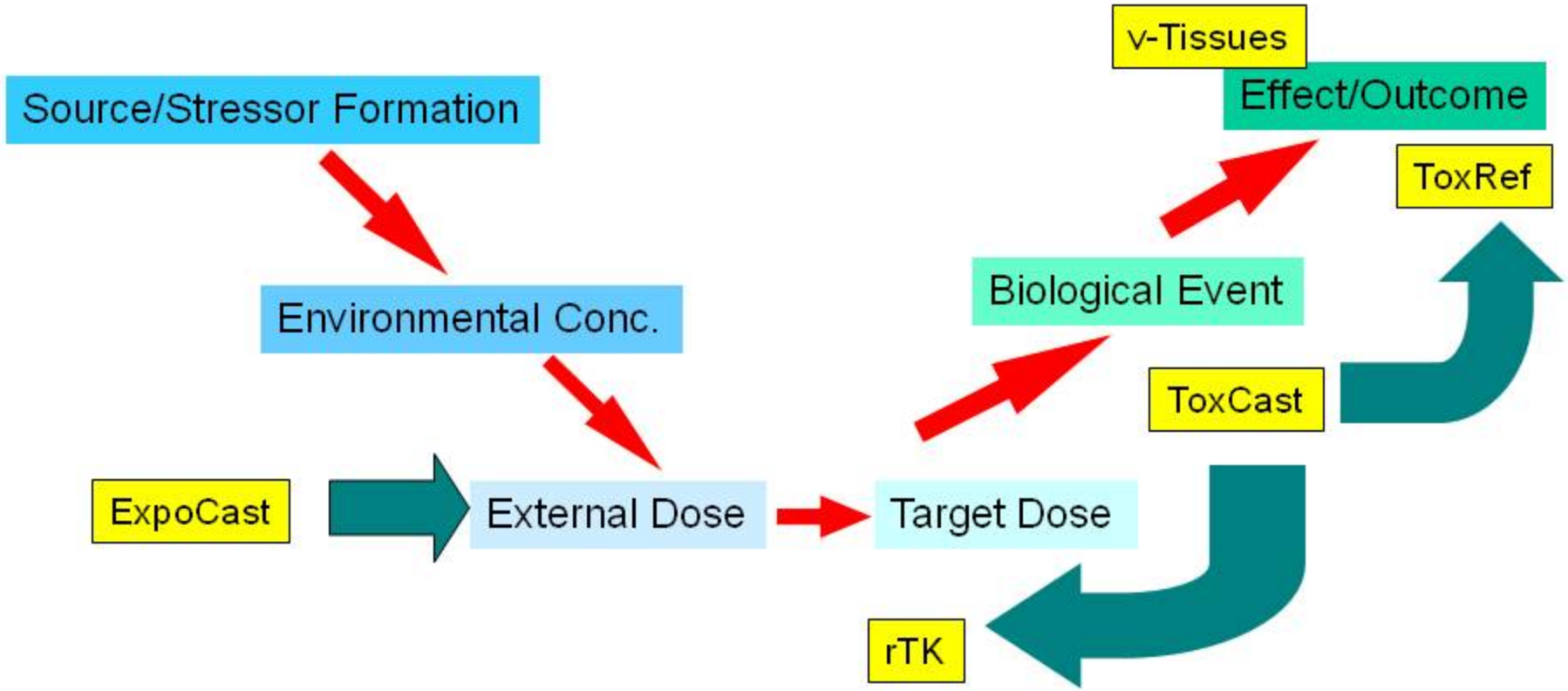
"...to integrate modern computing and information technology with molecular biology to improve Agency prioritization of data requirements and risk assessment of chemicals"

Decision Support Tools for High-Throughput Risk Assessment

Applying Computational Toxicology Along the Source to Outcome Continuum



ToxCast Prioritization Product Timeline

Phase	Number of Chemicals	Chemical Criteria	Purpose	Number of Assays	Cost per Chemical	Target Date
Ia	320	Data Rich (pesticides)	Signature Development	552	$20k	FY08
Ib	15	Nanomaterials	Pilot	166	$10K	FY09
IIa	>300	Data Rich Chemicals	Validation	>400	~$20-25k	FY09
IIb	>100	Known Human Toxicants	Extrapolation	>400	~$20-25k	FY09
IIc	>300	Expanded Structure and Use Diversity	Extension	>400	~$20-25k	FY10
IId	>12	Nanomaterials	PMN	>200	~$15-20K	FY09-10
III	Thousands	Data poor	Prediction and Prioritization	>300	~$15-20k	FY11-12

FY07 FY08 FY09 FY10 FY11 FY12

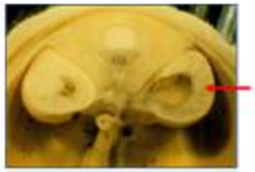
Proof of Concept

Verification/Extension

Reduce to Practice

Profiling developmental toxicity

***in vivo* endpoints** (target, description)
www.epa.gov/ncct/toxrefdb

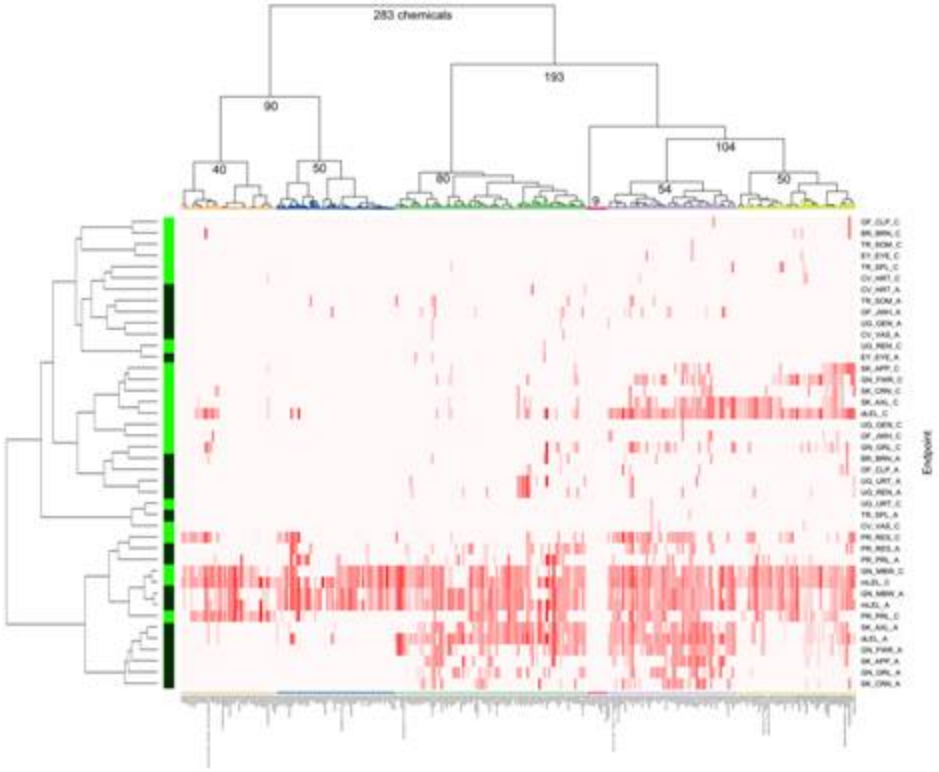


target: kidney
description: absent renal papilla
code: UG_REN_3.1060.5013

target: sternebra
description: incomplete ossification
code: SK_AXL_2.1099.5130

target: hindpaw
description: polydactyly (digit I)
code: SK_APP_2.1051.5234

Images from www.DevTox.org

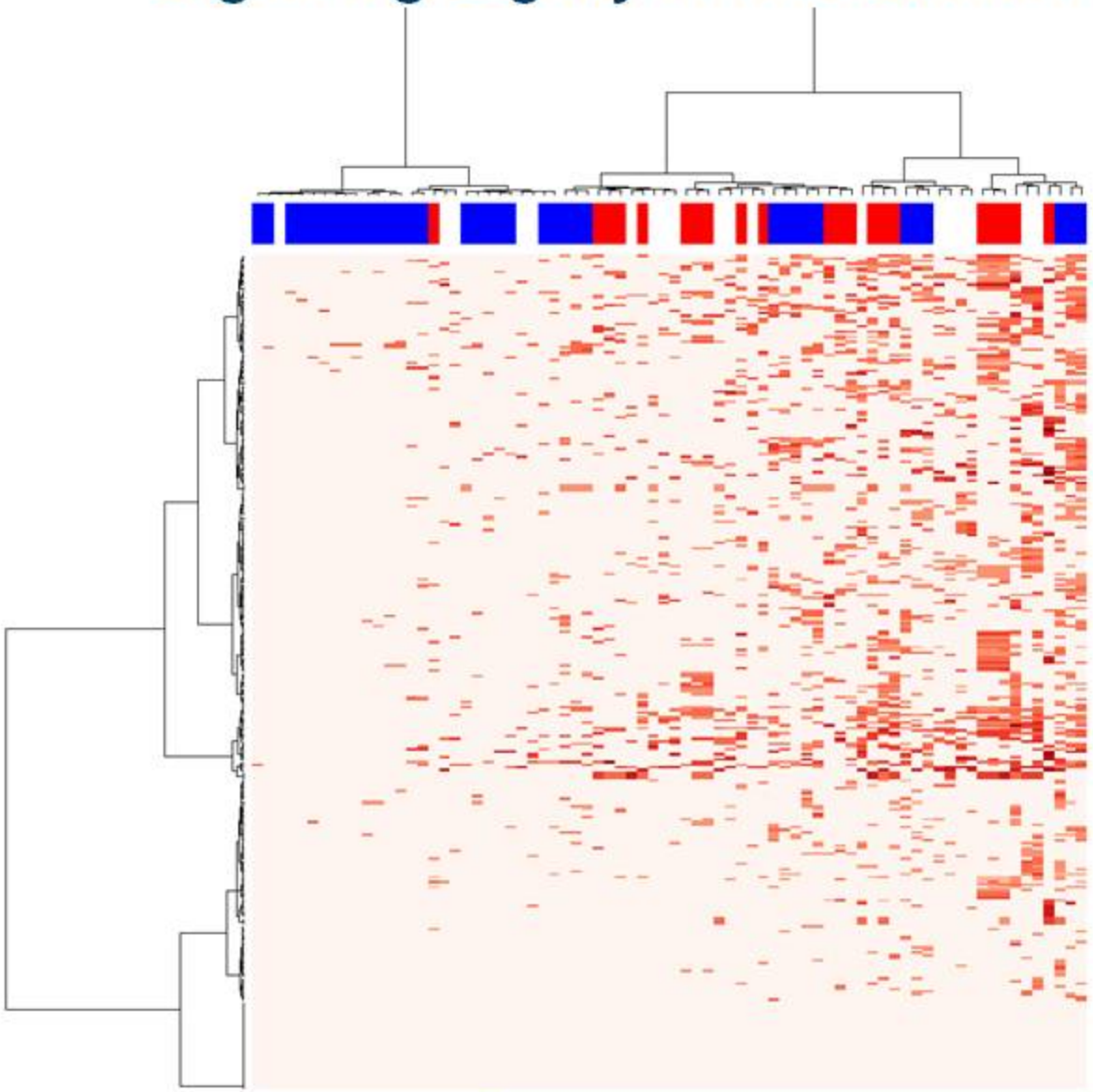


ToxRefDB 387 chemicals, 751 prenatal studies, 988 effects annotated (enhanced DevTox.org)

283 chemicals x 293 effects → 19 target systems from rat (■) and rabbit (■) studies

SOURCE: Knudsen et al. (2009) Reproductive Toxicology (in press) DOI 10.1016/j.reprotox.2009.03.016

Digitizing Legacy *in Vivo* Data in ToxRefDB



Chronic/Cancer
Multigenation
Developmental

Martin et al 2009a,b
Knudsen et al 2009

30 years and more than $2B worth of data

ToxCast Assays

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Biochemical Assays

- Protein families
  - GPCR
  - NR
  - Kinase
  - Phosphatase
  - Protease
  - Other enzyme
  - Ion channel
  - Transporter
- Assay formats
  - Radioligand binding
  - Enzyme activity
  - Co-activator recruitment

467 Endpoints

Cellular Assays

- Cell lines
  - HepG2 human hepatoblastoma
  - A549 human lung carcinoma
  - HEK 293 human embryonic kidney
- Primary cells
  - Human endothelial cells
  - Human monocytes
  - Human keratinocytes
  - Human fibroblasts
  - Human proximal tubule kidney cells
  - Human small airway epithelial cells
- Biotransformation competent cells
  - Primary rat hepatocytes
  - Primary human hepatocytes
- Assay formats
  - Cytotoxicity
  - Reporter gene
  - Gene expression
  - Biomarker production
  - High-content imaging for cellular phenotype

ToxCast *in vitro* data (467 assays)

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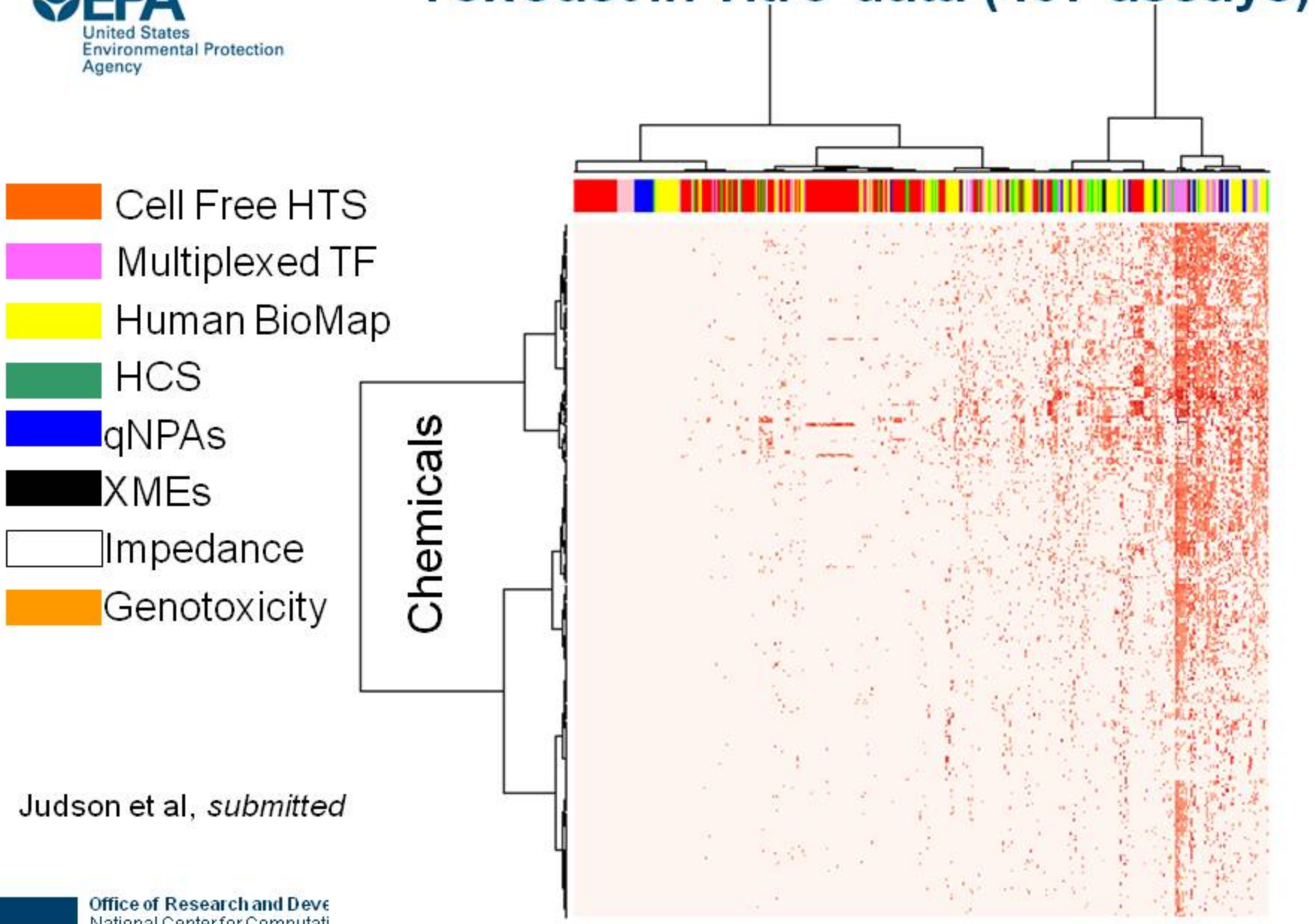
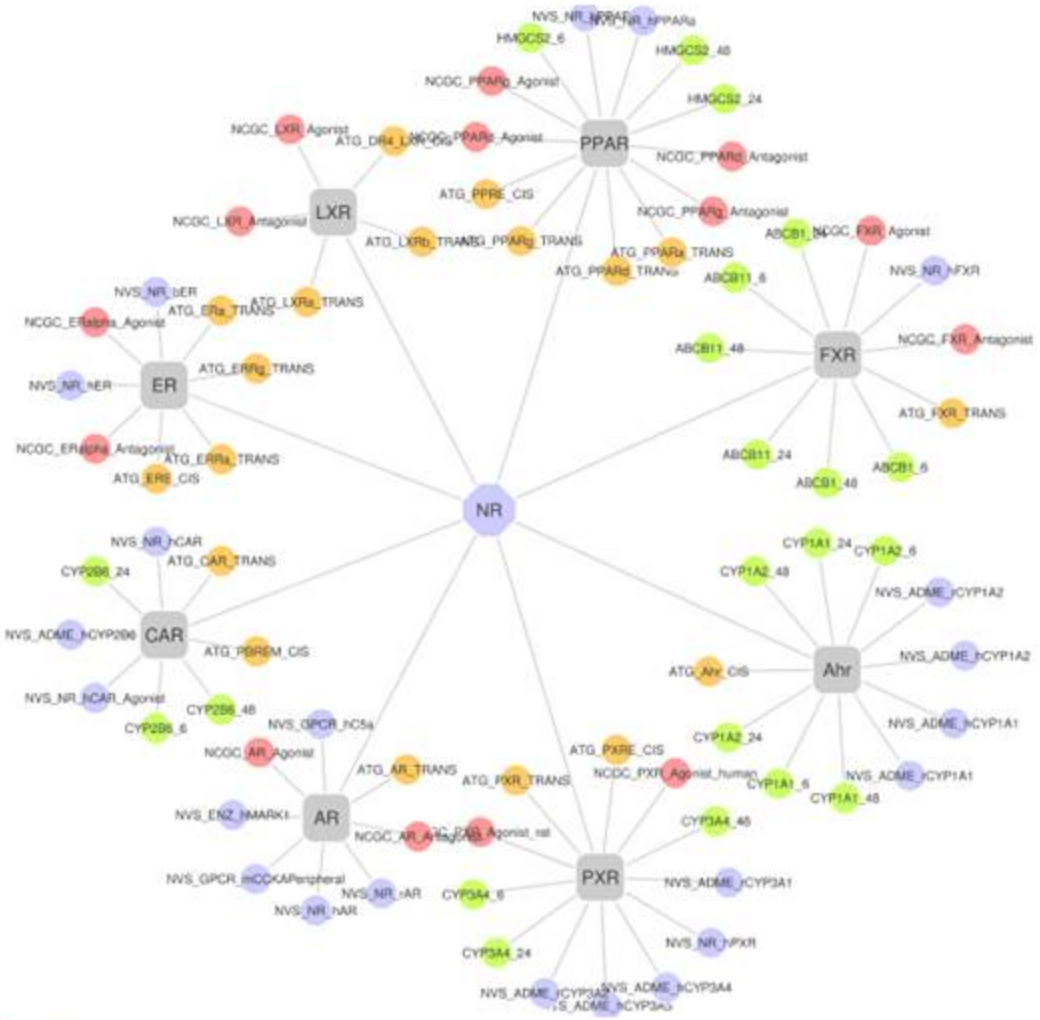


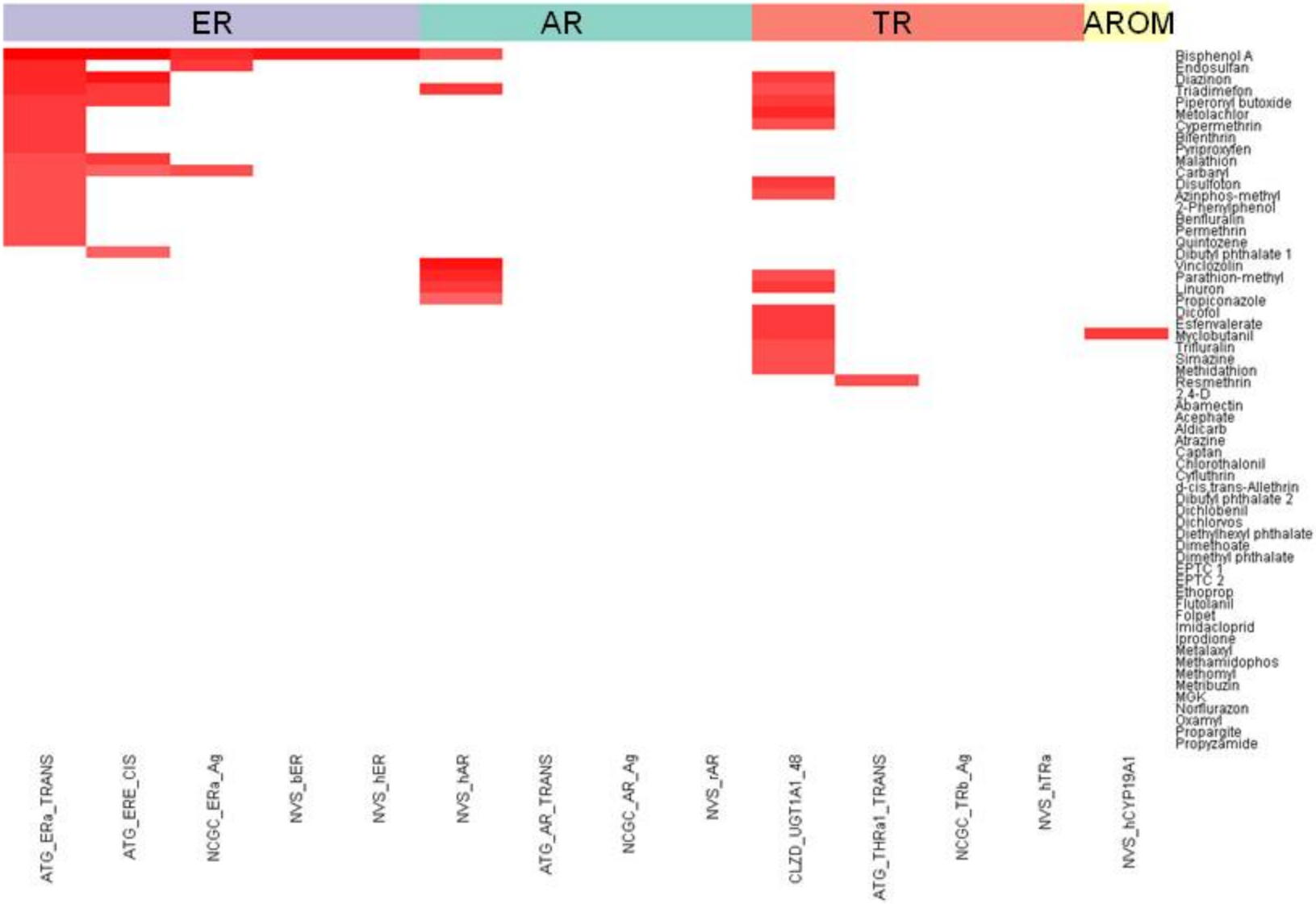
Judson et al, *submitted*

>200,000 dose response experiments

Multiple Assays per Endocrine Pathway



Molecular Targets in ToxCast for Endocrine Profiling



Sample Prioritization Rankings for EDCS

CHEM_NAME	EDSP	AR Priority	ER Priority	TR Priority	AROM Priority	Other Priority	OVERAL Priority
Bisphenol A	X	0.52	18.27	0.18	0.00	4.95	***23.22***
Myclobutanil	X	0.00	0.12	0.23	4.00	2.28	***6.62***
Parathion-methyl	X	0.52	0.63	0.23	0.00	2.27	***3.13***
Endosulfan	X	0.00	1.75	0.18	0.00	0.85	***2.60***
Azinphos-methyl	X	0.00	0.12	0.61	0.00	1.85	***2.57***
Carbaryl	X	0.00	1.75	0.56	0.00	0.33	***2.46***
Methidathion	X	0.00	0.30	0.41	0.00	1.83	***2.36***
Triadimefon	X	0.52	0.82	0.41	0.00	1.31	***2.36***
Piperonyl butoxide	X	0.00	0.30	0.41	0.00	1.71	***2.24***
Propiconazole	X	0.52	0.82	0.18	0.00	1.42	***2.24***
Malathion	X	0.00	0.30	0.18	0.00	1.82	***2.12***
Linuron	X	0.52	0.52	0.61	0.00	0.97	***2.10***
Vinclozolin	X	0.52	0.63	0.00	0.00	1.21	***1.84***

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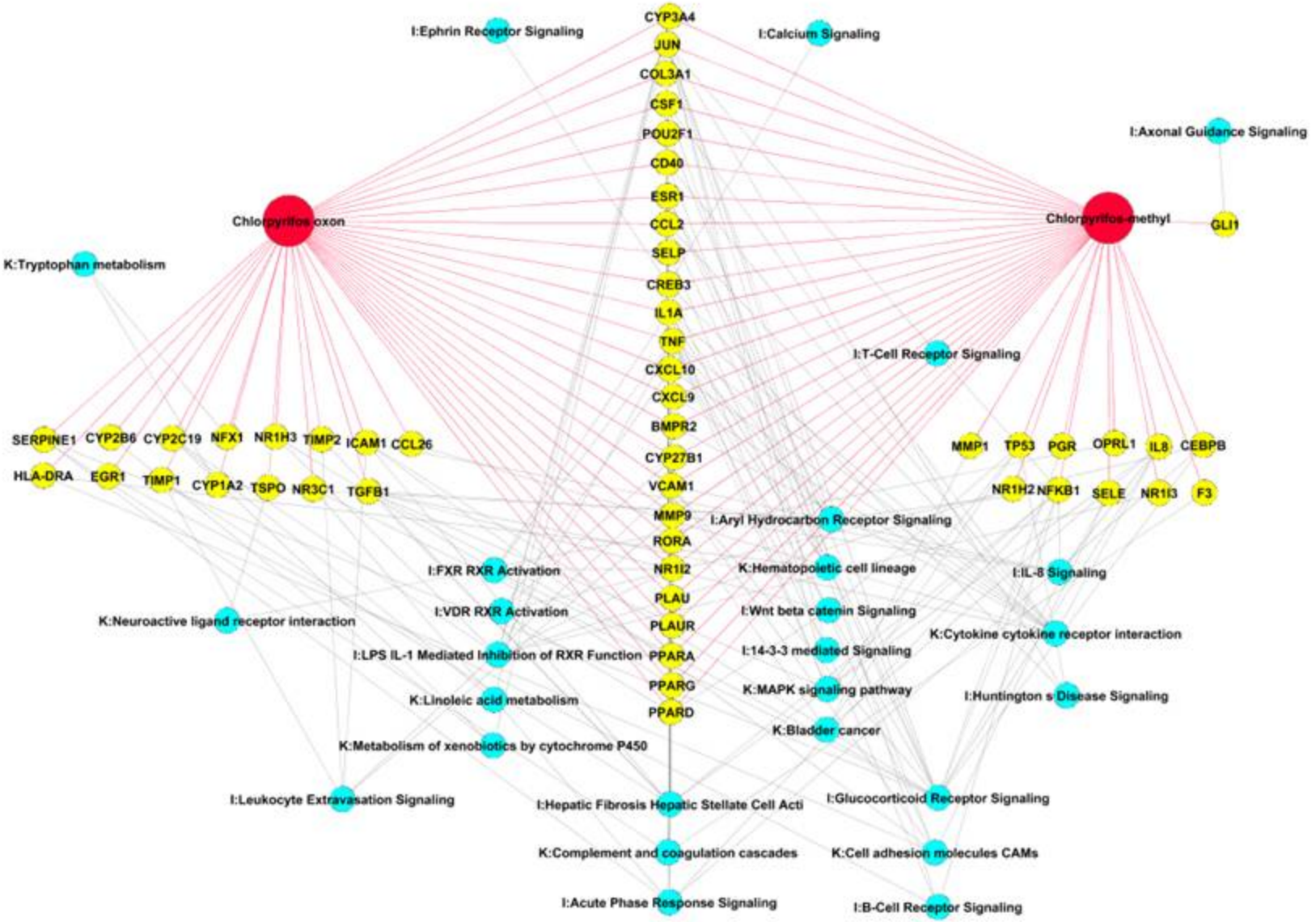
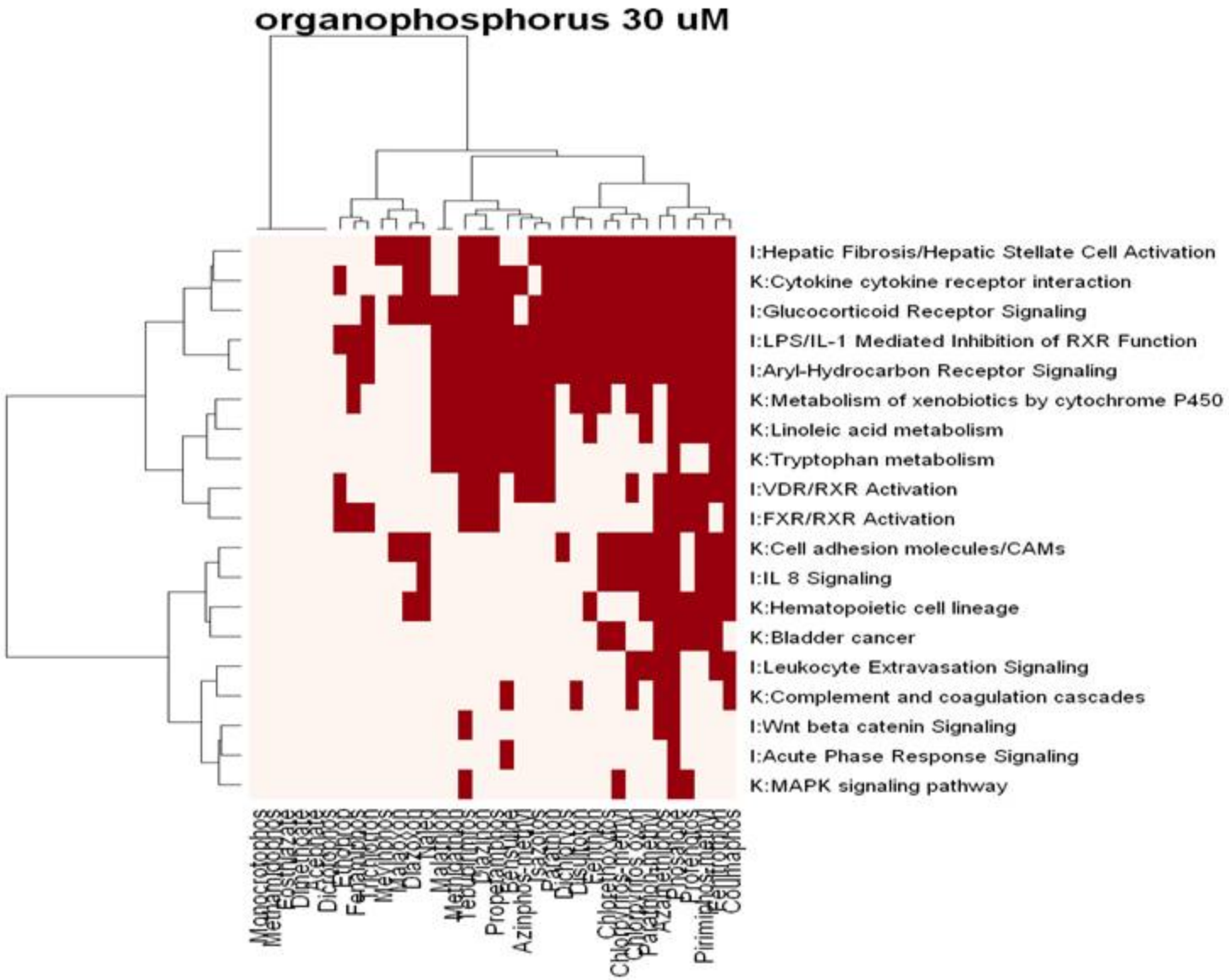
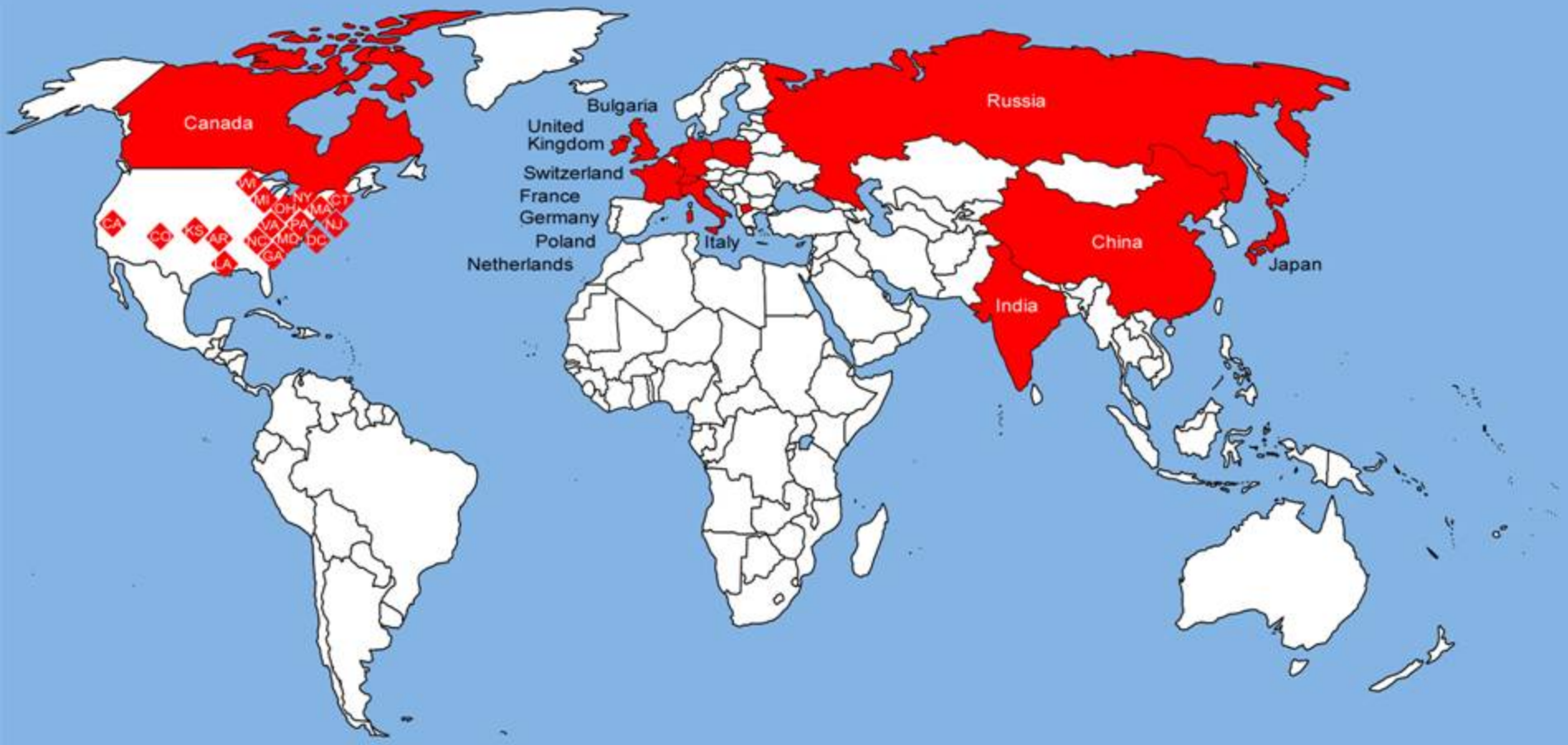
Pathway Targets for Chlorpyrifos



Pathway Hits for Organophosphates



TDAS 1 Data Analysis Partners



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Lessons Learned from Phase I

- High quality HTS data is obtainable
- A number of expected observations were found, as were a number of unexpected ones
- Multiple assays per biological pathway are important to include
- Many chemicals in the library interact with a number of targets
- The in vitro and in vivo data sets are complicated and will require extensive data analysis to determine optimal approaches
- Prioritization scores based on hazard potential are feasible
- Metabolism remains a challenge to incorporate in many assays
- Greater numbers of chemicals and assays are needed

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MATERIALS TRANSFER AGREEMENT

EPA:

U.S. Environmental Protection Agency (EPA)
Office of Research and Development (ORD)
National Center for Computational Toxicology (NCCT)

Pfizer:
Pfizer Inc, having a principal place of business at 235 East 42nd Street, New York, ("Pfizer") New York, 10017 and its Affiliates

WHEREAS the EPA wishes to obtain Pfizer Compounds to use in certain test assay panels, and whereas Pfizer wishes to have Pfizer Compounds evaluated on such test panels, the parties agree as follows:

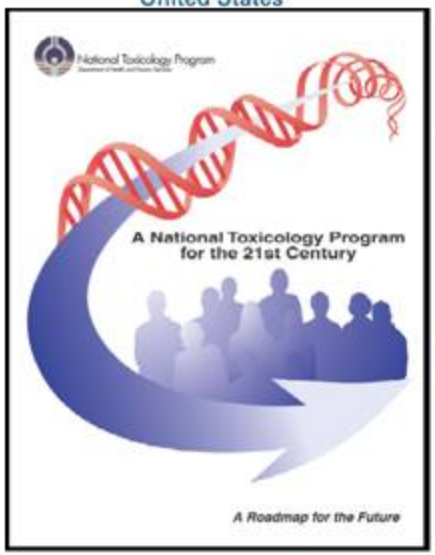
"Affiliate" means any corporation, firm partnership or other entity which directly or indirectly controls, is controlled by, or is under common control with either of the parties.

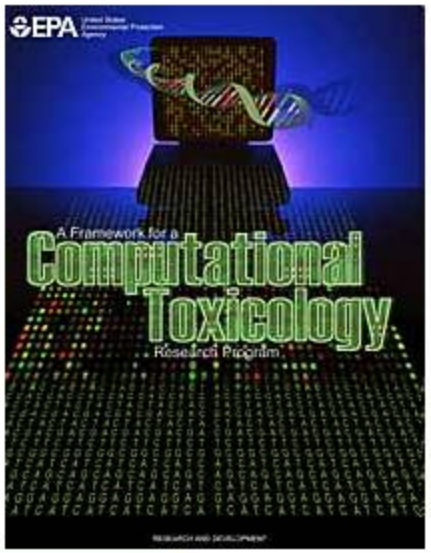
1. EPA agrees to receive Pfizer's compounds, listed in Exhibit B, in any form or any of its intermediates and derivatives ("Pfizer Compound"), in order to perform the research activities, further described in Exhibit A, and known as the "ToxCast™ Program."

2. The Pfizer Compounds:
   a. are the property of Pfizer and all existing rights including, without limitation, patent rights in or to the Pfizer Compounds will remain the property of the Pfizer.
   b. will be used with caution and for research purposes only, and shall not be used for research involving human subjects.
   c. will be used only by the EPA in the ToxCast™ Program described below, under suitable containment conditions.
   d. will not be used for screening, production or sale, for which a commercialization license may be required.

Both Pfizer and EPA agree to comply with all applicable laws, rules, guidelines and regulations applicable to the use, storage, shipping and the handling of the Pfizer Compounds and ToxCast™ Program.

Tox21 Community Development






POLICY FORUM

Transforming Environmental Health Protection

2004 **1.4k Library** 2006 **2.8k Library** 2008 **10k Library** 2010

2005

2007


THE NATIONAL ACADEMIES REPORT IN BRIEF

July 2007

Toxicity Testing in the 21st Century: A Vision and a Strategy

2009

Tox21

5M data points to date

Tox21 Working Groups

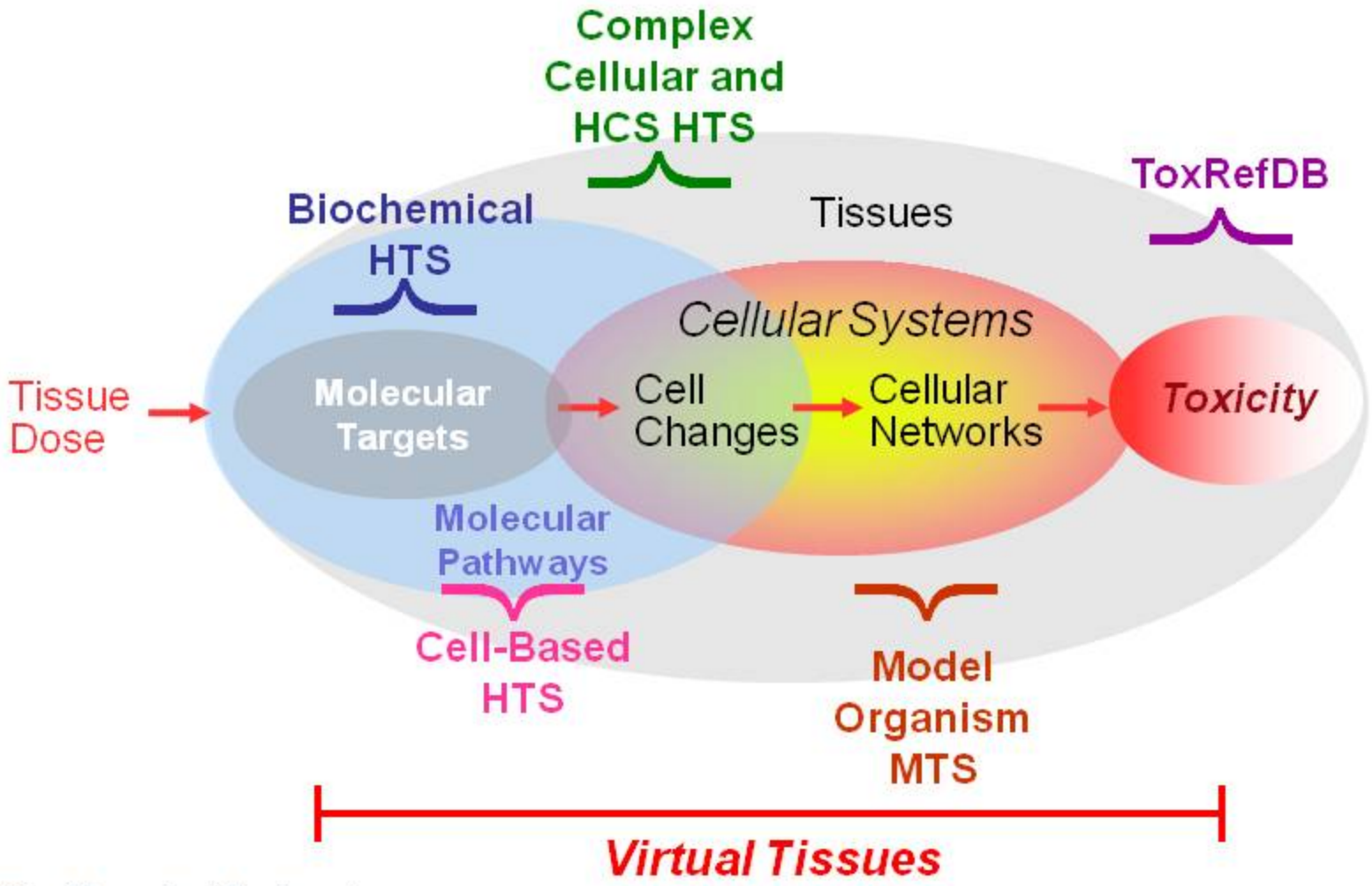
- **Pathways/Assays - K. Witt (NTP), K. Houck (EPA), M.□ Xia (NCGC)**
  - Identify key toxicity pathways/assays (with a focus on human cells) and prioritize assays for use
  - Identify assay gaps and consider methods for filling those gaps
  - Develop methods for incorporating hepatic metabolism into *in vitro* assays
  - Consider approaches for evaluating compound, pathway, and cell-to-cell interactions

- **Compounds - C. Smith (NTP), A. Richard (EPA), N. Southall (NCGC)**
  - Establish a library ~10,000 compounds with known structures for testing at the NCGC
  - Establish procedures for determining the identity, purity, and stability of each compound

- **Bioinformatics - K. Shockley (NTP), R. Judson (EPA), R. Huang (NCGC)**
  - Evaluate patterns of response and relationship to adverse health outcomes in experimental animals and humans
  - Evaluate consistency of response within assays and across related endpoints
  - *Make all data publicly accessible (PubChem, ACToR, CEBS)*

- **Targeted Testing - J. Bucher (NTP), S. Edwards (EPA), J. Inglese (NCGC)**
  - Prioritize substances for more complex testing, including the use of alternative assay platforms or species (e.g., *C. elegans*, zebrafish)

Predicting Human Toxicity: The Grand Challenge in Toxicology

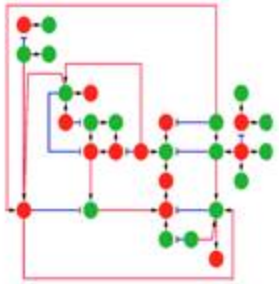


v-Liver™ Architecture

Assays	v-Liver Knowledgebase	v-Liver Simulator	Outcomes

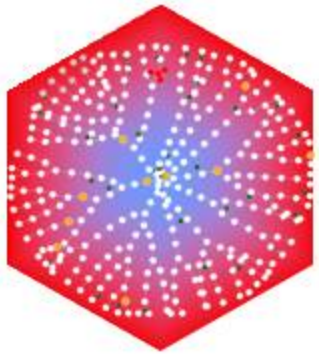
Env. Chems

ToxCast HTS, HCS *ex vivo*

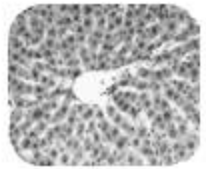


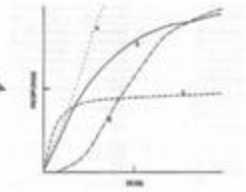
Molecular Events

Cell-Cell Events



Cell Sys. & Blood Flow





Cellular & Tissue Effects

The Future State: Using Hazard and Exposure Information for Assisting Design and Prioritizing Testing and Monitoring